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Review

Upper aero-digestive contamination by *Pseudomonas aeruginosa* and implications in Cystic FibrosisR. Rivas Caldas^{a,*}, S. Boisramé^{b,c,d,**}^a Laboratoire Universitaire de Biodiversité et d'Ecologie Microbienne (LUBEM-EA 3882), Université de Bretagne Occidentale, Université Européenne de Bretagne (UBO-UEB), 22 avenue C. Desmoulins, 29238 Brest Cedex, France^b Département de Chirurgie Orale, Pôle Organes des Sens, Centre Hospitalier Régional Universitaire (CHRU), 5 Av. Foch, 29609 Brest Cedex, France^c Laboratoire Universitaire de Biodiversité et d'Ecologie Microbienne (LUBEM-EA 3882), (UBO-UEB), 22 avenue C. Desmoulins, 29238 Brest Cedex, France^d CRCM, centre de Perharidy, Route de Perharidy, 29680 Roscoff, FranceReceived 22 November 2013; received in revised form 25 April 2014; accepted 29 April 2014
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Abstract

Background: Cystic Fibrosis (CF) is a severe genetic disorder that is common among the Caucasian population. Bacterial respiratory infections are the main cause of morbidity and mortality in CF patients. *Pseudomonas aeruginosa* is the main pathogen of lower airways (LAW) decline.**Method:** To understand chronic broncho-pulmonary colonization, a systematic review is conducted. The aim of our article is to identify the pathways of contamination in the upper aero-digestive tract.**Results:** A large number of articles report that *P. aeruginosa* is established first at nasopharyngeal sites. The vast majority of authors agree that the upper aero-digestive tract is the first location of colonization by *P. aeruginosa* and its presence appears to be predictive of subsequent broncho-pulmonary colonization.**Conclusion:** This review supports the possible involvement of the nasal and paranasal sinuses and oral cavity as means of contamination.

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Keywords: *Pseudomonas aeruginosa*; Cystic Fibrosis; Mouth; Paranasal sinuses; Nasal cavity

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1. Introduction

Cystic Fibrosis is one of the most common autosomal recessive lethal disorders affecting the Caucasian population. The rate in Brittany, France is 1/3300 births [1]. In 1974, the average age of death was 8 years due to a lack of appropriate treatment. The disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene on the long arm of chromosome 7, which codes for a transmembrane regulator protein. More than 1500 mutations have been reported to date [2]. *CFTR* mutations result in an abnormal performance of the cAMP-dependent chloride channel on the apical membrane of epithelial cells, manifested as dehydrated airway mucus, disordered cilia-motility and impaired mucociliary clearance [2–4]. The main clinical manifestations are characterized by malabsorption, chronic rhinosinusitis and LAW infections. The natural history of this disease includes acquisition through time of bacterial species. During early childhood, the broncho-pulmonary infections are due to *Haemophilus influenzae* and *Staphylococcus aureus*. As the patient grows older, pathogenic Gram-negative bacteria like *Achromobacter xylosoxidans*, *Burkholderia cepacia* complex and especially *Pseudomonas aeruginosa* are more frequently seen. *P. aeruginosa* is a turning point in the respiratory disease and its prevalence increases with age [5]. Before arriving to the lungs, these respiratory pathogens cross different anatomical areas like the nose and paranasal sinuses on the one hand, and the oral cavity on the other converging in the oropharynx. The pharynx is a transition zone and an intersection between the respiratory tract and digestive tract.

Initially, *P. aeruginosa* colonizes the lungs intermittently, and after rapid adaptation, it turns into chronic infection [2,6]. Chronic airway infection with *P. aeruginosa* and bronchiectasis are the major causes of morbidity and subsequent mortality in CF patients [7,8]. Once established in the lungs, this organism is extremely difficult to eradicate.

P. aeruginosa and especially the mucoid strains are responsible for lung decline in CF patients. There is a vicious circle due to local inflammation caused by this bacterium that causes lesions in CF patients generating a loss of respiratory function [9].

This ubiquitous aerobic bacterium is noted for its environmental versatility. In contrast to many environmental bacteria, *P. aeruginosa* rarely causes infection in healthy individuals but it has a remarkable capacity to cause disease in susceptible hosts. This bacterium is an opportunistic pathogen associated with respiratory tract infections, causing nosocomial infections in hospitalized and immunocompromised patients who are mechanically ventilated [6].

P. aeruginosa possesses several factors that contribute to colonization and chronic infections, in particular the capacity to form biofilm composed of exopolysaccharides as well as host mucin and water up to 97%. The initial step is the biofilm adhesion to a surface. In CF, epithelial mucus accumulation facilitates this process. This biofilm is found in different parts of the body. Colonized lungs with presence of biofilm confer to this bacterium a large resistance to antibiotics and reduce the immune response of patients. Bacteria populations organized in biofilm are more difficult to eradicate [6].

Often, this bacterium changes its morphology. It becomes immobile, decreases the production of virulent factors, changes its lipopolysaccharide (LPS) structure [3] and becomes a mucoid strain [6,10]. In addition, *P. aeruginosa* isolates in chronically infected patients are phenotypically different from those isolates in other patients (in ventilator-associated pneumonia patients and patients with acute respiratory failure) or from those in the environment. It can produce several pigments, a blue-green pigment pyocyanin, a fluorescent yellow-green pigment and a brown-red pigment pyomelanin.

Others factors implicated in colonization are surface components, the polar pili for eukaryotic cell attachment; flagella expression in initial stages; and the type III secretion system, the major virulence factor that allows bacteria to inject toxins into host cells. One of the secreted proteins is ExoU that causes damage to cellular membranes and rapid necrotic death. Another factor is *P. aeruginosa* Quorum Sensing (cell-to-cell signaling), a density-dependent system that coordinates gene expression by the production of small diffusible molecules such as acyl homoserine lactones (AHL). Some of interconnecting genes are implicated in biofilm formation, making conditions favorable for bacterial multiplication and survival and formation of structured communities.

Persistence of *P. aeruginosa* colonization is due to the genetic flexibility provided by its large genome [6]. The large proportion of hypermutable strains favors adaptation. This hypermutable character is generated when the rate of spontaneous mutation is 100–1000 times higher than usual.

Mutation development favors bacterial persistence with a higher resistance to antibiotics because of the large amount of antimicrobial treatments in CF patients. The presence of 43% hypermutable *P. aeruginosa* in CF patients has been demonstrated [10].

In VAP, *P. aeruginosa* causes a mortality rate of 34–48%, due to damage of the epithelium associated with endotracheal intubation that favors bacterial colonization of the endotracheal tube and upper respiratory tract. In immunosuppressed patients, transplant recipients, cancer and neutropenia, the risk of acquiring *P. aeruginosa* is increased, with a mortality rate of 40% in *P. aeruginosa* associated pneumonia.

The aim of our study is to systematically review the different pathways of *P. aeruginosa* in the upper aero-digestive tract implicated in chronic LAW colonization to better understand the stages of colonization.

2. Methods

2.1. Search strategy

In November 2013, we conducted a literature review searching in electronic databases and scanning reference lists in PubMed to identify studies with *P. aeruginosa* human colonization data. We searched in English, Spanish and French languages. We used the keywords, Mesh terms, synonyms: *P. aeruginosa*, CF, nose, paranasal sinus, oral cavity, saliva, upper airways (UAW) and LAW to find studies, case reports relating association of these search terms.

2.2. Selection of studies

The titles and abstracts of all identified studies were screened and reviewed to identify potential studies or case reports with *P. aeruginosa* impacting on health. Articles with inaccessible abstracts and conclusions not concordant with article titles were not used. Only articles helping to understand *P. aeruginosa* implication in upper aero-digestive tract and its potential consequences in CF patients were considered.

2.3. Data analysis

The studies were grouped by category according to the following inclusion criteria:

- Cystic Fibrosis
- *P. aeruginosa*
- *P. aeruginosa* and nose and paranasal sinuses
- *P. aeruginosa* and oral cavity (saliva, teeth, periodontum)
- Data published between 1954 and 2013.

The exclusion criteria were as follows:

- Cystic Fibrosis on animal models
- Cystic Fibrosis and other bacterial pathogens.

Our aim was to identify the origin of *P. aeruginosa* in Cystic Fibrosis: nose, paranasal sinuses and oral cavity.

3. Results

3.1. Article research

Data analysis was performed by ranging articles every ten years with selected keywords. We found 18,446 CF articles between 1954 and 2013 and 1057 including *P. aeruginosa* keywords. We focused on the theme of our research and grouped according to the year and the number of keyword articles. A

graph (Fig. 1) showing evolution of publications is reported throughout the assessed years.

For a better understanding, we compiled in a table (Table 1) the most relevant articles and identified the topic (CF or not), type of study, number of patients included, methods used and conclusions.

3.2. *P. aeruginosa*, nose and paranasal sinuses

Several studies discuss paranasal sinuses and bacterial colonization. Paranasal sinuses are composed of four paired air-containing cavities around the nasal cavity: maxillary sinuses on either side, frontal sinuses above ocular cavities, ethmoid sinuses between the eyes, and behind the ethmoid sinus, and the sphenoid sinuses.

Several authors report an increased incidence of paranasal sinus inflammation associated with hypoplasia of these air-containing cavities [11].

Foremost, Woodworth et al. showed that sinus hypoplasia is correlated to the CF genotype. In their retrospective study, 31 of 45 CF patients had sinus surgery. And, in this cohort, 25 homozygous F508del-*CFTR* had a significantly increased frequency of under-developed frontal, maxillary, and sphenoid sinuses when compared with other mutations [12].

Eggesbo et al. analyzed, in a case control study, the volume of sphenoid sinuses in 96 CF patients with median age of 19 years versus 130 control population with median age 32 years by X-ray computed tomography. They concluded that CF patients with two confirmed mutations had significantly smaller sphenoid sinuses than in controls [13]. In another study in 2002, Eggesbo et al. including 108 CF patients (median 18 years) and 79 controls (median 31 years) conclude that reduced mucociliary clearance, due to high mucus viscosity results in different sinonasal inflammatory patterns such as higher rate of sinonasal polyposis, sphenoethmoid recess and ostiomeatal complex patterns in CF patients [14].

Sinonasal anatomy in CF patients usually differs from non CF patients. Chronic rhinosinusitis (CRS) and nasal polyposis are hallmarks in the CF population [11,15]. Nasal polyposis is a

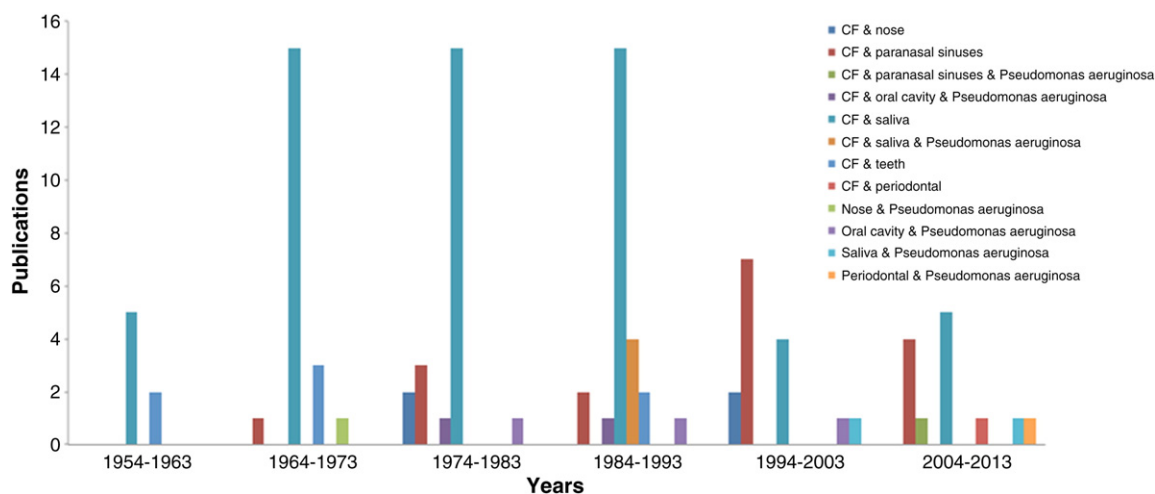


Fig. 1. Evolution of the number of international publications by topic between 1954 and 2013.

Table 1
Literature data on UAW manifestations of CF patients.

Ref.	Researchers	Interest	Year	Study type	Number of patients	Sampling and/or methods	Results and relevant conclusions
4	Digoy G.P. et al.	CF paranasal sinuses	2012	Retrospective chart study	51 CF children (age < 18 years)	Cultures from the maxillary sinuses obtained during endoscopic sinus surgery	<i>Staphylococcus aureus</i> was the most common isolate found in the maxillary sinuses in children with CF, followed by <i>Pseudomonas aeruginosa</i> and <i>Haemophilus influenzae</i>
11	Gentile V.G. et al.	Sinusitis in CF	1996	Prospective longitudinal study	19 CF children	Computed tomography of paranasal sinuses	There are 2 distinct patterns of sinus disease: chronic sinusitis and polyposis
12	Woodworth B.A. et al.	CF paranasal sinuses	2007	Retrospective review	45 CF patients (median 32 years)	Computed tomography of paranasal sinuses	CF patients homozygous for the delta F508 mutation have a greater incidence of hypoplastic or underdeveloped sinuses
13	Eggesbo H.B. et al.	CF sinuses	1999	Transversal case control study	96 CF patients (median 19 years) vs 130 controls subjects (median 32 years) with inflammatory sinonasal disease	Computed tomography of paranasal and sphenoid sinuses	Hypoplasia of the sphenoid sinuses is a characteristic finding in CF patients.
14	Eggesbo H.B. et al.	CF paranasal sinuses	2002	Prospective transversal case control study	108 CF patients (median 18 years) vs. 79 controls subjects (median 31 years) with paranasal sinus diseases	Computed tomography of paranasal sinuses	CF patients had more widespread sinonasal inflammatory changes and more advanced disease for each sinus. Most of them displayed polyposis and sphenoethmoid recess patterns. Inflammatory paranasal sinuses is caused by the impaired mucociliary clearance in CF
15	Anaes K.	CF bacterial sinusitis	2013	Prospective longitudinal study	nonspecified	Functional endoscopic sinus surgery (FESS), culture methods	Sinuses can be a focus for initial lung colonization and infection, sinus inflammation facilitates persistence of bacteria.
17	Berkhout M.C. et al.	UAW bacteriology in CF	2013	Cross sectional study	104 CF patients	UAW samples obtained by nasal lavage and middle meatal swabs. LAW samples obtained by expectorated sputum or cough swabs. Cultural methods	UAW and LAW cultures showed one or more concordant microorganism in 50% of the patients. <i>Pseudomonas aeruginosa</i> was most frequently cultured from the UAW.
18	Mainz J.G. et al.	Clonal relatedness between CF UAW and CF LAW isolates	2009	Prospective transversal study	182 CF patients (median 17 years)	UAW sampled by nasal lavage, and LAW by expectorated sputum and deep nasal swabs. 16 SNPs typing	23 of 24 CF patients carried identical <i>Pseudomonas aeruginosa</i> SNP genotypes and 31 of 36 CF patients carried <i>Staphylococcus aureus spa</i> in UAW and LAW, suggesting that the UAW play a role as a reservoir in CF
19	Shapiro E.D. et al.	Sinuses bacteriology in CF	1982	Observational study	20 CF patients (median 15 years)	Ultrasound, radiography, and transantral sinus aspiration. Cultural methods	The organisms most commonly recovered from the sinus aspirates were <i>Pseudomonas aeruginosa</i> , <i>Haemophilus influenzae</i> , <i>streptococci</i> and anaerobes. There was no association between the sinonasal bacterial species and the predominant bacterial species in the nasopharyngeal, throat, or sputum culture.
20	Franché G.L. et al.	Nasal bacteriology in CF	2007	Cross sectional prospective study	23 CF children (age 5 years)	Middle meatus aspiration by unendoscope. Cultural methods	The presence of <i>Pseudomonas aeruginosa</i> was observed in 18.18% of the positive cultures, and <i>Staphylococcus aureus</i> was observed in 27.28%.

(continued on next page)

Table 1 (continued)

Ref.	Researchers	Interest	Year	Study type	Number of patients	Sampling and/or methods	Results and relevant conclusions
21	Muhlebach M.S. et al.	Sinuses bacteriology in CF	2006	Prospective study	25 CF patients (median 9,5 years)	Sampling was made using a sterile rayon culturette during sinus surgery, oropharyngeal swabs and bronchoalveolar lavage. Cultural methods and PFGE	The great majority of the middle meatus aspirates of the patients with cystic fibrosis were negative. Bacterial sinus infection was present in 96%, caused by <i>Staphylococcus aureus</i> (49%), <i>Pseudomonas aeruginosa</i> (42%), and <i>Haemophilus influenzae</i> (22%). Bacterial species were the same in sinus and OP or BALF samples of 12 patients of these bacteria 83% showed
22	Mainz J.G. et al.	<i>Pseudomonas aeruginosa</i> in CF lungs	2012	Case reports	2 CF patients (20 years) with double lung transplantation (LTX) due to <i>Pseudomonas aeruginosa</i> related pulmonary destruction	Nasal lavage analyzed by custom-made microarray	It demonstrated persistence of identical <i>Pseudomonas aeruginosa</i> genotypes UAW prior to and after LTX underlining risks of descending colonization of transplanted lungs with <i>Pseudomonas aeruginosa</i>
23	Hansen SK et al.	<i>Pseudomonas aeruginosa</i> in CF paranasal sinuses	2012	Longitudinal study	46 CF children	Expectoration, endolaryngeal suction, BAL and functional endoscopic surgery	Paranasal sinuses constitute an important niche for the colonizing bacteria in many patients
26	Charlson E.S. et al.	Lung bacteriology	2012	Prospective transversal study	6 patients undergoing clinical bronchoscopy	Oral wash of the oropharynx and bronchoalveolar lavage. PCR amplification and pyrosequencing	BAL sample contains substantial oropharyngeal bacteria. These sampling methods allow identification of microbes that can replicate in the lung despite the background due to oropharyngeal microbes derived from aspiration and bronchoscopic carry-over.
27	Scannapieco F.A. et al.	Bacterial respiratory pathogens in the oral cavity in care unit patients	2009	Prospective longitudinal case–control study	146 mechanically-ventilated patients (median 48 years): (47 patients under chlorhexidine treatment 1 day; 50 patients under chlorhexidine treatment 2 day vs. 49 controls)	Dental plaque samples were collected with a sterile stainless steel curette or sterile swab and tracheal samples with a sterile suction catheter at admission and every 48 h until discharge. Cultural methods	While decontamination of the oral cavity with chlorhexidine did not reduce the total number of potential respiratory pathogens, it did reduce the number of <i>S. aureus</i> in dental plaque of trauma intensive care patients.
29	Heo S.M. et al.	Bacterial respiratory pathogens in the AUW et LAW in hospitalized patients	2008	Prospective longitudinal study	30 patients with suspected Ventilator-associated pneumonia (VAP)	Supragingival dental plaque samples were collected with a sterile curette or sterile swab, tracheal samples with a sterile suction catheter and bronchoalveolar lavage at admission and every 48 h until discharge. PFGE and MLST	Plaque and lung respiratory pathogens are often genetically indistinguishable; this provides convincing evidence that dental plaque serves as an important reservoir for respiratory pathogens
30	Perkins S.D. et al.	Ways of lungs colonization by oral pathogens	2010	Retrospective transversal study	8 mechanically-ventilated patients in intensive care unit (median 17 years)	Bacterial biofilm was collected to endotracheal tubes (ET). Bacterial community identification by quantitative PCR and gene surveys targeting 16S rRNA genes	Study shows that potentially pathogenic bacteria existed in ET tube biofilms within 24 h incubation. In the ET tube that was in place for 23 d, 95% of the sequences belonged to <i>Pseudomonas aeruginosa</i> . Harboring such pathogens in ET biofilms may increase the chance of VAP
31	Rams T.E. et al.	Oral cavity as source of CF lungs infections	1990	Cross sectional study	506 subjects with advanced adult periodontitis	Subgingival samples were collected with paper points. Culture method and identification with API STAPH Tract	Higher prevalence of <i>Staphylococcus aureus</i> in the 50% of subjects. Subgingival plaque could be a source of this bacterium for descending infection.

32	Worlitzsch D. et al.	Oral cavity as source of CF lungs infections	2010	Prospective transversal case–control study	11 CF patients vs. 11 control	Biochemical identification methods of sputum samples only in CF patients, and periodontal pockets	In 4 of 9 CF sputum identical facultative anaerobes (<i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Burkholderia cepacia</i>) were found in periodontal pockets and in sputum. In all patients identical obligate anaerobic strains were found in both compartments
33	Komiyama K et al.	<i>Pseudomonas aeruginosa</i> in CF oral cavity	1985	Observational study	31 CF patients	Quantitative cultures of buccal mucosa, dorsum of the tongue, saliva, dental plaque	The dorsum of the tongue gave the highest yield of <i>Pseudomonas aeruginosa</i> followed by the buccal mucosa, saliva, and dental plaque. Oral colonization by the mucoid variants of <i>Pseudomonas aeruginosa</i> may lead to further colonization in the LAW and subsequent
34	Zuanazzi D. et al.	Bacterial respiratory pathogens in the oral cavity in hospitalized patients	2010	Retrospective transversal study	30 patients undergoing myocardium revascularisation surgery	Saliva and biofilm samples obtained in pre- and post-operative phases. Bacterial detection by PCR and culture	<i>Staphylococcus</i> spp. (85.7%) was the most prevalent bacteria, followed by <i>Pseudomonas</i> spp. (83.8%), and <i>Acinetobacter</i> spp. (53.3%). The oral cavity of hospitalised patients harbors high frequencies of bacterial respiratory pathogens, supporting its potential role as a reservoir.
35	Fourrier F. et al.	Bacteria of dental plaque in intensive care patients	1998	Prospective longitudinal study	57 patients in MICU	Quantitative cultures of dental plaque, nasal secretions, salivary and tracheal aspirates, and urine at admission (day 0) and every 5 day until death or discharge.	A high bacterial concordance was found between dental plaque and tracheal aspirate cultures and between salivary and dental plaque cultures. In 6 of 21 cases of nosocomial infection, responsible pathogen was isolated from dental plaque.
36	Scannapieco F.A. et al.	Bacteria of dental plaque in intensive care patients	1992	Prospective study	34 medical Intensive Care Unit (MICU) patients vs 25 preventive dentistry clinic patients	Quantitative cultures of dental plaque and buccal mucosa	Bacteria commonly causing nosocomial pneumonia colonize the dental plaque and oral mucosa of intensive
38	Abe S. et al.	Bacterial respiratory pathogens in the oral cavity in daily nursing care patients	2001	Case control study	54 subjects (median 75.3 years) in daily nursing care vs. 21 controls (median 69.5 years) and 22 controls (median 25.4 years)	Sterile phosphate buffered saline lavage during 10 s. Culture methods, PCR detection and PFGE.	care patients. Dental plaque may be an important reservoir of these pathogens in medical ICU patients Professional oral health care for elderly patients requiring daily nursing plays a significant role in reducing the numbers of potential respiratory pathogens in the oral cavity.

manifestation in CF with a peak incidence between 5 and 13 years. Polyposis may be responsible for generating a favorable environment for bacteria pathogen colonization and the exaggerated immune response dominated by neutrophils [16].

CRS is a chronic inflammation that is related to anatomical causes (deviated septum, turbinate hypertrophy), environmental factors (smoking, humidity), inflammatory and infectious causes (bacterial, viral and fungal infections, allergies, nasal polyposis), to immunosuppression and to the presence of nasal sinus tumors obstructing the natural drainage.

Even if functional factors are unknown, CFTR channels might be involved in the development of these diseases. CFTR Cl^- ion transport controls the hydration of mucosal surfaces and promotes effective mucociliary clearance. Association of viscous mucus, dysfunctional ionic transport in epithelial cell, damage of mucociliary drainage and inflamed tissues could explain the mechanical obstruction of the sinus ostia.

This CRS affects the quality of life and may also contribute to pulmonary exacerbations with the possibility of the microbial infection descending into CF patient's lungs [17,18].

Although morphological abnormalities of the paranasal sinuses are present in nearly all CF patients, the microbiology of their sinuses has gained more attention over the last years.

Shapiro et al., was one of the first authors to publish concerning bacteriology of maxillary sinuses in CF patients in 1982. 34 aspirations were conducted on 20 CF patients. 95% had at least one positive culture ($\geq 10^4$ colony-forming units/ml). The most common organisms were *P. aeruginosa*, *H. influenzae*, *Streptococci* and anaerobes. No association was found between bacterial species in nasopharyngeal, throat or sputum culture [19].

In another study, Franche et al. didn't find a relation between sputum and middle meatus aspiration cultures. In positive cultures, they found the presence of *P. aeruginosa* in 18.2% and *S. aureus* in 27.3%. Moreover, a statistically significant relation was revealed between rhinorrhea and positive cultures of middle meatus aspiration [20].

Recently, Digoy et al. reported that *S. aureus* was the most common bacteria in cultures from the maxillary sinuses obtained during endoscopic surgery on 51 CF children (with mean age of 9) followed by *P. aeruginosa* and *H. influenzae* [4]. Comparing with the Shapiro et al. [19] study which showed that *P. aeruginosa* is the most common isolate in 15 years old CF patients, we can submit the hypothesis that bacterial pathogens are frequently acquired in an age-dependent sequence. Other bacterial pathogens were retrieved: *Staphylococcus (non-aureus)*, *B. cepacia*, *Stenotrophomonas maltophilia*, *Streptococcus pneumoniae*, and *Escherichia coli* [4].

Muhlebach et al. reported that *P. aeruginosa* is the most prevalent bacteria in sinus aspirations in association with anaerobic bacteria [21].

Several studies revealed the relation between sinonasal bacterial colonization and subsequent lung colonization that supports the idea of bacterial spread from sinuses to LAW [17,19,21].

Muhlebach et al. studied 45 paired sinus-bronchoalveolar lavage fluid (BALF) cultures of 31 CF patients (median 8, 2 years; range of 3.2–18.7 years). Phenotypically identical cultures were

analyzed by Pulsed-Field Gel Electrophoresis (PFGE). They revealed that *P. aeruginosa* is the most prevalent bacteria in older CF children's sinus cultures and BALF compared to younger CF children. Interestingly they showed the presence of 2 *P. aeruginosa* isolates phenotypically different with the same PFGE pattern, and in 83% of the samples analyzed they showed identical genotypes in UAW and LAW cultures. This fact could be explained by a descending infection [21].

Recently, Mainz et al. in 2012, studied the UAW's role as a reservoir and gateway for acquisition of opportunistic bacteria, as *P. aeruginosa* and *S. aureus*, and subsequent LAW microbial colonization in CF. They assessed a study including 182 CF patients (median 17 years) (63 chronically colonized with *P. aeruginosa*). UAW were sampled by nasal lavage and LAW by sputa collection and deep throat swabs. UAW and LAW were positive for *P. aeruginosa* in 29 and 57 cases respectively, for a total of 63 patients. Available 16 SNPs typing showed 95.8% genetic identity between UAW and LAW *P. aeruginosa* isolates. Finally, the authors concluded that CF UAW are a reservoir for descending infection to the lung [18].

In a second study, Mainz et al., in 2 CF post lung-transplanted adults showed that transplanted donor lungs became infected with identical *P. aeruginosa* clones previously found in the explanted lungs, with a high proportion of identical phenotypic and genetic strains in the sinuses. This finding demonstrates persistence of identical *P. aeruginosa* isolates in UAW and the subsequent lung colonization via UAW descending infection, supporting results of their first study [22].

Hansen et al., corroborate that paranasal sinuses are a potential niche for *P. aeruginosa* clones that allow bacteria to adapt and subsequently cause chronic lung infection [23].

Later, Berkhout et al. demonstrated, in their study of 104 patients, that *P. aeruginosa* was the most prevalent pathogenic bacteria in UAW cultures (48.1%). This pathogen was found in LAW despite its eradication in UAW (three successive negative cultures in six months). Even though this bacterium was eradicated in UAW, it was to be a pathogen in LAW [17]. UAW are less exposed to antibiotics, host immune cells and airflow than LAW, making an ideal reservoir for bacterial growth through the heterogeneous conditions [2].

Cramer et al. revealed in a study of 955 *P. aeruginosa* isolates, that the *P. aeruginosa* population is genotypically composed by a few ubiquitous clones (environment and human disease habitats contain twenty most common clones). In CF patients, five of these 20 most common clones are found [3].

So, detection and recognition of sino-nasal flora are useful to know the potential role of this UAW reservoir for bacterial pathogens in order to treat it rapidly and prevent chronic lung colonization.

3.3. *P. aeruginosa* and oral cavity

The oral cavity is an anatomical complex region comprising different tissues. It is composed of a maxilla and a mandible where teeth are found, the supporting tissues of the tooth called periodontal tissues. The inner faces of the cheeks come on each

side of the jaw and in the center is the tongue. These tissues are bathed in saliva.

The mouth is continuously colonized by hundreds of different micro-organisms on different surfaces: teeth, dorsum of the tongue, and oral mucosa that constitute oral biofilm. Dental plaque consists of at least 800 bacterial species [24] and serves as a permanent reservoir of microorganisms that are not usually in the normal oral microbiota such as potential respiratory pathogens (PRPs).

Moreover, there is modification of the oral microbiota depending on the state of health of patients. For example, *S. aureus*, *S. pneumoniae*, *Acinetobacter baumannii*, *H. influenzae* and *P. aeruginosa* become predominant and constitute a more aggressive flora in critically ill patients [25]. Before entering the broncho-pulmonary tree, it is clear that the oral cavity and oropharynx are first colonized by these PRPs.

Very few articles were reported for *P. aeruginosa* presence in the oral cavity of CF patients. This section aims to show that some anatomical sites or physiological conditions (periodontal tissues, dental plaque and saliva) will be a potential niche for PRPs including *P. aeruginosa*.

Several studies of intensive-care unit and hospitalized patients reveal that dental plaque is an important reservoir for PRPs, as *S. aureus* and *P. aeruginosa*. Supragingival biofilm samples show the presence of bacteria able to cause nosocomial pneumonia, caused by aspiration of oropharyngeal or UAW bacteria [26]. Moreover, dental plaque bacteria can be released in the saliva and then, aspirated into the LAW causing infections [27].

For patients who receive mechanical ventilation in intensive care units, it's well known that the lack of oral hygiene by mechanical tooth-brushing allows increased microbiota diversity and quantity. Some papers report the impact of 0.12% chlorhexidine gluconate (CHX) oral rinse, as Scannapieco et al., for the decontamination of the oral cavity. This oral rinse inhibits the viability of planktonic bacteria found mostly in saliva. However, its action is very limited on the biofilm and in the Scannapieco et al. study, CHX did not reduce the numbers of Gram-negative bacteria in oral biofilm [27].

Abe et al. determined the effectiveness of oral care performed by health professionals in reducing aspiration pneumonia. Three groups constitute the cohorts: 54 patients >65 years requiring daily care, 21 patients >65 years in good health, and 22 patients <30 years in good health. They examined the prevalence of respiratory pathogens in samples of gargle by culture and PCR. In the group of elderly people in institutions, microbes found in descending order are *C. albicans*, *S. pneumoniae*, *Staphylococcus* spp., *S. aureus* methicillin-resistant, and *P. aeruginosa*. The numbers of *C. albicans* cells recovered in samples from elderly subjects were significantly higher than those recovered from the healthy young group. Elderly patients needing daily care and receiving professional oral health care had lower prevalence and cell numbers of *C. albicans* than did the elderly patients without such oral care. This study showed that professional oral health care in elderly requiring daily nursing care reduced the cell numbers of PRPs [28].

Heo et al. demonstrated in 2008 that PRPs taken in dental plaque are genetically identical to strains from BALF in patients

who were under mechanical ventilation with VAP. Other findings demonstrated strong correlations between oropharyngeal colonization and positive BALF samples [29].

Different authors explain that in VAP, the endotracheal tube, which is an artificial surface, to which bacteria adhere and form colonies resulting in the formation of biofilm, bypasses the anatomical barriers (between the trachea and oropharynx) and facilitates the transport of bacteria from the oral cavity into the lungs. It is recognized that *Streptococcus* spp. belonging to the normal oral microflora promote recruitment of bacteria in dental plaque through production of extracellular glucans and permit coaggregation to other microbes. So, they start the colonization process [30].

In addition to this aspect of coaggregation, disease of the supporting tissues called periodontal disease, influences lung infections. To enhance this point of view, cross sectional studies on oral *S. aureus* prevalence determined a higher prevalence rate of this bacterium in the 50% of patients with periodontal disease in comparison to healthy people [31].

Worlitzsch et al. investigated the role of periodontal pockets as a possible source for CF lung infections in a case control study with 22 patients. Facultative anaerobes such as *P. aeruginosa*, *S. aureus* and *B. cepacia* and obligate anaerobes such as *Prevotella* spp., *Propionibacterium* spp. and *Actinomyces* spp. were found respectively for 44.4% and for 100% both in periodontal pockets and sputum. So, they conclude that the periodontal pocket is a source of broncho-pulmonary infection [32].

In addition, *P. aeruginosa* was investigated at different sites of the oral cavity (dorsum of the tongue, dental plaque, and saliva) and sputum from 31 CF patients. In their study, the dorsum of the tongue is where *P. aeruginosa* is the most frequently detected followed by oral mucosa, saliva and dental plaque. For the authors, oral colonization by *P. aeruginosa* may lead to future UAW colonization [33].

Regarding the study of Charlson et al. that analyzed oral wash (OW) and BALF in 6 patients with lungs disease, BALF and OW samples in 5 patients presented the same types of bacteria, with a high prevalence of *Prevotella* spp. and *Streptococcus* spp. One of lung transplanted patients showed a higher prevalence of *Streptococcus* spp. and *Rothia* spp. in OW; and BALF sample was dominated by *P. aeruginosa* [26]. This data suggests that there is first a change in the microbial community composition among patients colonized and non-colonized with *P. aeruginosa*. And secondly, the microbial community in OW and BALF changes in relation to presence of *P. aeruginosa*.

According to saliva contamination, Zuanazzi et al. demonstrated the presence in high proportion of PRPs in oral cavity of hospitalized patients. They evaluated the prevalence of oral colonization by PRPs (*Acinetobacter* spp., *Pseudomonas* spp., *S. aureus* and *Dialister pneumosintes*) in 30 patients who underwent cardiovascular surgery. An oral clinical exam was performed at the beginning of this research protocol. Saliva and biofilm samples were obtained before and after surgery (after orotracheal extubation).

Thirteen were edentulous (ED) and 17 were dentate (DE), with moderate chronic periodontitis. The most prevalent bacteria

in saliva, in descending order, were *Staphylococcus* spp., *Pseudomonas* spp., and *Acinetobacter* spp. In plaque samples, DE with >14 teeth showed a significantly higher prevalence of *Pseudomonas* spp. than individuals with ≤14 teeth. A strong correlation between the presence of *Acinetobacter* spp. and *Pseudomonas* spp. was observed [34].

It appears that mouth tissue and the presence of humidity are portals of entry for respiratory pathogens and their arrival in the LAW. Fourrier et al. showed that bacteria causing pneumonia are first found in dental plaque [35] and in addition Scannapieco et al. revealed that the use of antibiotics modifies oral microbiote and favors oral colonization with respiratory pathogens [36]. This fact can explain bacterial colonization in CF patients, who are continuously treated by antibiotics.

4. Conclusion

This literature review highlights the presence of PRPs like *P. aeruginosa* in the nose and paranasal sinuses but also in the oral cavity and oropharynx.

Their presence constitutes a continuing risk of chronic lung recontamination. Paranasal, oral and oropharyngeal microbiote could play a possible role in the pathogenicity of *P. aeruginosa* and contribute to its establishment in the LAW. However, mechanisms of pathogen acquisition and persistence in CF LAW are not well elucidated, thereby limiting the prevention of lung descending infection.

Unfortunately, there is a lack of longitudinal studies to clarify whether *P. aeruginosa* is a transient member of the oral flora in chronically broncho-pulmonary colonization in CF patients. Future studies are needed in this CF population, to learn about the prevalence of *P. aeruginosa*, the genotype concordance with *P. aeruginosa* lung colonization on the one hand and on the other hand to understand the presence of oral bacteria like periodontal bacteria in the LAW in these patients and their interactions with this principal pathogen.

Considering the possible role of the upper aero-digestive airway as a reservoir for lung infections with pathogens such as *P. aeruginosa*, protocols of sampling for bacteriological analysis should be optimized to prevent passage of oral and nasal bacteria into the LAW. Early bacterial detection in the upper aero-digestive airway and subsequent adaptive treatment for eradication could improve the success in preventing LAW colonization. Future investigations of sampling by non-invasive methods of the upper aero-digestive airway (like collection of saliva) would be interesting.

Conflict of interest

The authors declare that they have no conflict of interests.

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